

Supplementary Information

Therapeutic Effects of *Acer Palmatum* Thumb. leave extract (KIOM-2015E) on Benzalkonium Chloride-Induced Dry Eye in a Mouse Model

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Supplementary Figure S1. Schematic of experimental design

Supplementary Figure S2. Changes in ocular surface at 2 weeks and 4 weeks in 0.2% BAC treatment.

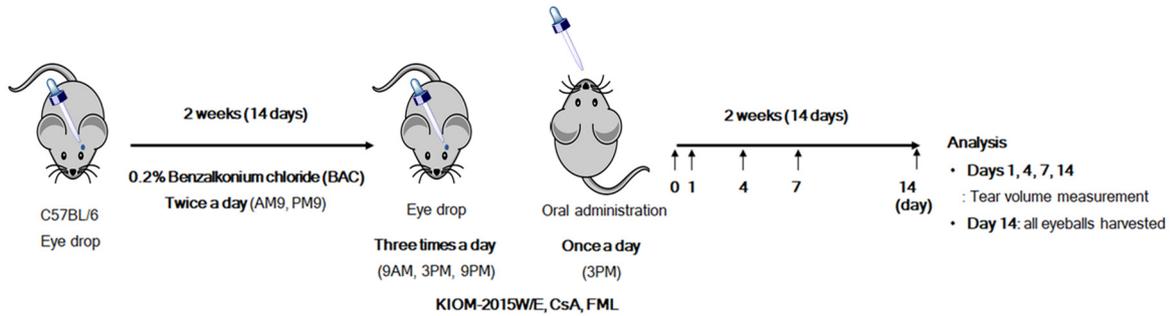
Supplementary Figure S3. Changes in ocular surface at 2 weeks and 4 weeks in 0.2% BAC treatment.

Supplementary Figure S4. Source data of western blot analysis shown in Figure 2A and 2B

Supplementary Figure S5. Quantification tables.

Figure S1

(A)



(B)

Conditions (groups)	1 week	2 week	3 week	4 week
Normal (n = 11)	Non-treatment			
0.2% BAC only (n=11)	0.2% BAC		0.2% BAC	
0.2% BAC + PBS vehicle (E.D.) (n = 8)	0.2% BAC		E.D. Normal saline	
0.2% BAC + KIOM-2015EW1 mg/ml (E.D.) (n = 8)	0.2% BAC		E.D. KIOM-2015EW	
0.2% BAC + KIOM-2015EE 0.5 mg/ml (E.D.) (n = 8)	0.2% BAC		E.D. KIOM-2015EE	
0.2% BAC + KIOM-2015EE 1 mg/ml (E.D.) (n = 8)	0.2% BAC		E.D. KIOM-2015EE	
0.2% BAC + KIOM-2015EE 100 mg/kg (P.O.) (n = 8)	0.2% BAC		P.O. KIOM-2015EE	
0.2% BAC + CsA 0.5mg/ml (E.D.) (n = 8)	0.2% BAC		E.D. T-sporin	
0.2% BAC + FML 1mg/ml (E.D.) (n = 8)	0.2% BAC		E.D. Fumelone	

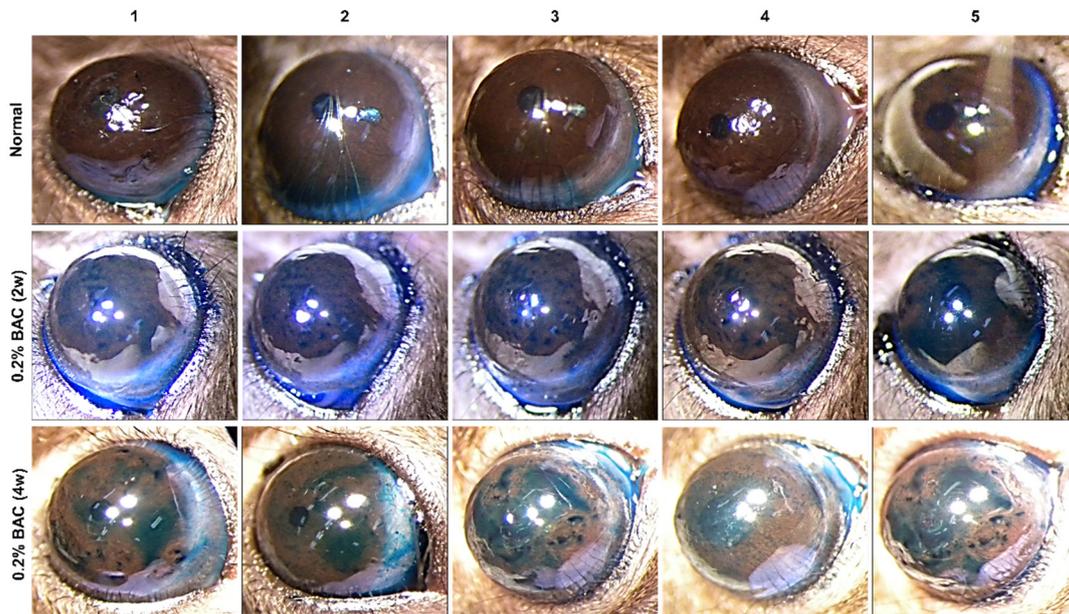
0.2% BAC : twice a day (AM 9:00, PM 9:00), 5 µl/eye rop

Drug treatment : three times a day (AM 9:00, PM 1:00, PM9:00), 5 µl/eye drop
once in a day (PM 1:00), 100 µl/oral administration

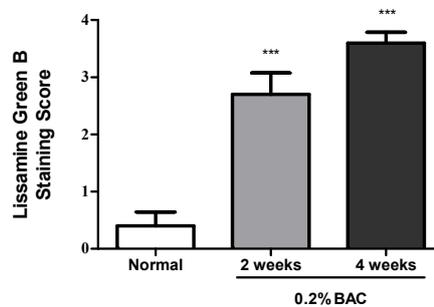
Supplementary Figure S1. Schematic of experimental design. Schematic of the experimental protocol of benzalkonium chloride (BAC)-induced DED model and drug application (A), and tabulated (B).

Figure S2

(A)



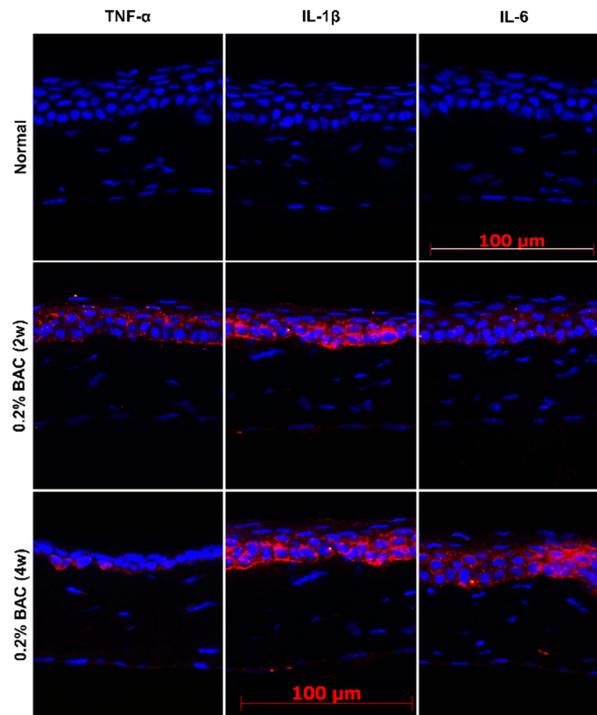
(B)



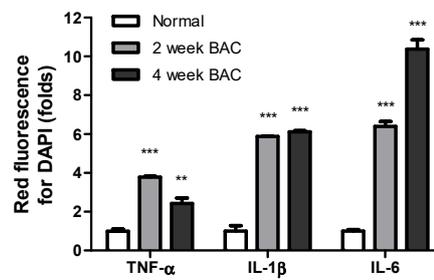
Supplementary Figure S2. Changes in ocular surface at 2 weeks and 4 weeks in 0.2% BAC treatment. (A) Representative images of corneal epithelium integrity including lissamine green B staining at 2 and 4 weeks of BAC treatment (n = 5). (B) Change in lissamine green B staining score relative to treatment level in BAC treatment. Statistical significance was analyzed by performing one-way ANOVA with Turkey's test using GraphPad Prism. Differences were considered statistically significant as follows: ***P<0.001 vs. Normal. BAC; benzalkonium chloride, 2w; 2 week, 4w; 4 week.

Figure S3

(A)



(B)



Supplementary Figure S3. Changes in ocular surface at 2 weeks and 4 weeks in 0.2% BAC treatment. (A)

Representative images for corneal cytokines immunofluorescence, merged with DAPI (n = 3). Scale bar, 100 μ m.

(B) The density of cells positively stained for fluorescence staining in the cornea tissues. Quantification of red-fluorescence density was performed using the ImageJ software program. These experiments were performed in triplicate, and the results are the average of 3 independent experiments. The results of the bar graph are the averages of 3 independent experiments. Statistical significance was analyzed by performing one-way ANOVA

with Turkey's test using GraphPad Prism. Differences were considered statistically significant as follows:
***P<0.001, **P<0.01 vs. Normal. BAC; benzalkonium chloride.

Figure S4. Source data of western blot analysis shown in Figure 5A.

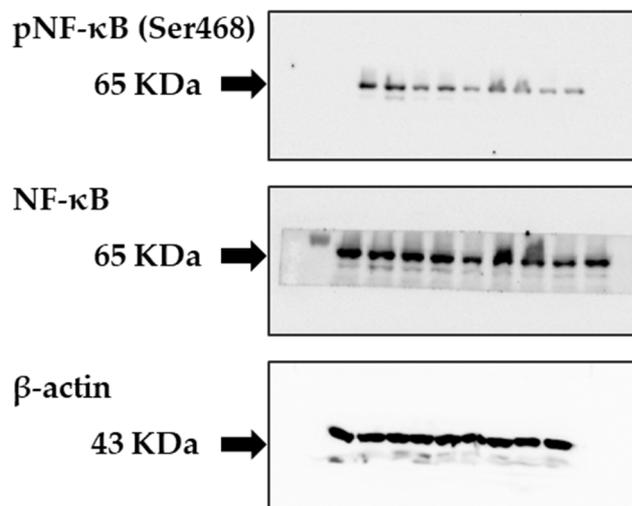


Figure S5

(A)

Tear production (mm)									
Day	Normal	0.2% BAC	+ PBS vehicle	+ EW 1 mg/mL	+ EE 0.5 mg/mL	+ EE 1 mg/mL	+ EE 100 mg/kg	+ CsA 0.5 mg/mL	+ FML 1 mg/mL
Mean±SD									
0D	4.50±0.51	2.61±0.76	-	-	-	-	-	-	-
1D	4.42±1.85	2.13±1.26	2.33±0.81	2.46±1.52	3.16±1.15	3.22±1.48	3.00±1.65	2.16±0.88	3.95±1.60
4D	4.54±2.61	2.25±0.91	2.69±1.27	6.07±2.39	7.76±0.74	7.56±1.55	5.84±1.80	3.26±1.41	6.83±1.52
7D	4.97±1.39	2.53±1.02	5.48±1.38	6.96±2.16	7.95±1.42	7.97±0.80	5.85±1.26	6.99±1.42	7.36±1.09
14D	5.21±0.92	2.57±0.52	6.69±1.79	8.25±2.82	10.62±1.46	10.33±2.43	8.08±1.57	6.47±1.26	7.46±2.09

(B)

Lissamine green B staining score			
Group	Mean	Min	Max
Normal	0.67	0	1
0.2% BAC	3.67	3	4
+ PBS vehicle	1.67	1	2
+ EW 1 mg/mL	1.00	1	1
+ EE 0.5 mg/mL	0.67	0	1
+ EE 1 mg/mL	0.33	0	1
+ EE 100 mg/kg	1.33	1	2
+ CsA 0.5 mg/mL	0.83	0.5	1
+ FML 1 mg/mL	0.67	0.5	1

(C)

Groups	Fluorescence analysis		
	Intensity (mean±SD, %)		
	TNF- α	IL-1 β	IL-6
Normal	9.26±1.28	12.81±1.77	7.22±0.60
0.2% BAC	25.51±2.60	61.35±2.74	62.22±3.98
+ PBS vehicle	13.29±1.75	20.72±2.16	21.93±3.97
+ EW 1 mg/mL	12.32±0.59	20.25±1.27	16.94±0.36
+ EE 0.5 mg/mL	6.53±0.18	14.98±1.04	14.69±0.97
+ EE 1 mg/mL	5.60±1.00	15.43±1.78	11.00±0.90

+ EE 100 mg/kg	10.87±1.57	21.17±2.29	19.17±5.07
+ CsA 0.5 mg/mL	7.85±0.61	20.43±2.47	9.52±0.82
+ FML 1 mg/mL	7.20±1.16	21.28±2.31	13.35±2.29

(D)

Groups	Relative mRNA expression analysis		
	Expression (mean±SD, fold)		
	TNF- α	IL-1 β	IL-6
Normal	1	1	1
0.2% BAC	1.11±0.05	1.12±0.01	1.14±0.05
+ PBS vehicle	0.99±0.00	1.10±0.01	1.10±0.01
+ EW 1 mg/mL	0.87±0.09	1.00±0.02	1.00±0.02
+ EE 0.5 mg/mL	0.84±0.05	0.97±0.00	0.97±0.01
+ EE 1 mg/mL	0.83±0.03	0.96±0.02	0.96±0.02
+ EE 100 mg/kg	0.95±0.05	0.98±0.04	0.98±0.04
+ CsA 0.5 mg/mL	0.87±0.09	0.97±0.03	0.97±0.03
+ FML 1 mg/mL	0.86±0.09	0.96±0.02	0.96±0.02

(E)

The corneal epithelial thickness									
Groups	Normal	0.2% BAC	+ PBS vehicle	+ EW 1 mg/mL	+ EE 0.5 mg/mL	+ EE 1 mg/mL	+ EE 100 mg/kg	+ CsA 0.5 mg/mL	+ FML 1 mg/mL
Fold (mean±SD)	1±0.03	0.62±0.02	0.87±0.09	0.91±0.06	0.99±0.06	0.98±0.07	1.09±0.08	1.13±0.10	0.95±0.02

Supplementary Figure S5. Quantification tables. (A) Tear production measurement with phenol-red cotton thread method. (B) The change of lissamine green B staining scores. (C) Fluorescence intensity analysis of immunofluorescence staining. (D) Analysis of TNF- α , IL-1 β and IL-6 mRNA expression levels in BAC-induced dry eye mouse model by quantitative real time PCR (qRT-PCR). Average results of 3 independent experiments. Values are presented as mean±SD. (E) Analysis of corneal epithelial thickness in BAC-induced dry eye mouse model. Five photographs were randomly selected in each group, and the corneal thickness was measured using the ImageJ program (pixel value). And then the respective values were expressed as folds to normal, and the five values were averaged.